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Research Paper

Potential Application of Consortium Microbe from Sea Cucumber Intestinal Symbiont as Preservatives for Vaname Shrimp

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Abstract
Vaname shrimp is one of the most profitable export commodities in Indonesia. However, the shrimp meat undergoes rapid quality degradation when shipped without any preservatives. This is an issue since the preservatives commonly found on the market are formalin-based. This study aims to discover a natural preservative solution by utilizing microbes. The objective of the study is to discover natural preservatives made of symbiont microbe in sea cucumber's intestinal organ by antimicrobial activity screening. Sea cucumber samples were collected from Bandengan waters of Jepara. There are 3 symbiotic microbe form intestinal cucumber that can inhibit the growth from *Bacillus cereus* and *Pseudomonas aeruginosa*. These three microbes are *Listeria sp.*, *Staphylococcus sp.*, and *Rothia sp.* Consequently, tested microbial samples were prepared into a consortium microbe and were tested further as a preservative agent for shrimp with a positive control parameter (cooling). The observations conducted in this study include organoleptic properties, acidity, total colony, proximate test (protein, water, ash, fat, and carbohydrate contents), and Total Volatile Base-Nitrogen (TVBN). The results found 3 active isolates are synergic one to each other as a bacterial consortium. Acidity test of sample extract treatment measured a pH of 7.44, compared to the non-treatment result of 7.14. Organoleptic test results of the shrimp indicated that the shrimp was acceptable for consumption. Proximate test of the treatment did not show a significant difference compared to the positive control treatment. Total colony and TVBN test on treated samples resulted in 48 x 105 CFU/ml and 39.62 mgN% respectively, whereas a similar test on non-treatment sample showed 119 x 105 CFU/ml and 45.31 mgN% respectively. It was concluded that the extract of sea cucumber symbiont microbe consortium showed potency in preventing meat quality degradation in shrimp, although treatment by freezing still produces a better result.

Keywords
Microbial Symbiont, Preservative, Sea Cucumber

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1. INTRODUCTION

Food decay is known as food degradation in normal storage (Buckle et al., 2007). According toBarus et al. (2017), one factor in marine food decay was caused by bacteria with the growth of bacteria will cause physical and chemical changes of the food. Ma'ruf (2012), stated that microorganism such as *P. aeruginosa* dan *B. cereus* had cause fish and other marine food. In this case, one of the important export commodity in Indonesia with the risk of decay is a product of vanname shrimp (Wijayanto et al., 2017).

Dealing with preservation in marine food in general such as frozen, smoking, radiation or food preservative additives. But this food preservation (radiation and frozen) will have a high operational cause. So that people will prefer to use low-cost synthetic preservative such as formalin (Goon, 2014). The use of formalin will have a very bad effect on a human. Formalin will cause irritation and inflammation in the mouth and esophagus, chest or stomach pain and in most savior condition is kidney failure. Therefore the option use of natural food preservation will be needed (Yulisa et al., 2014).

The marine microbe is one of potential resource for bioactive component commercialization. Microorganism with association with a marine organism such sea cucumber will be able to synthesize secondary metabolite in the host organism (Pringgenies, 2010). According to Ma'ruf et al. (2014), sea cucumber genus of Holothuria had been proven as potential antibacterial ability from its bioactive component. Antibacterial from Sea cucumber had been effectively had proven as an antibacterial compound for *B. cereus* and *P. aeruginosa*. Three symbiotic bacteria from intestine

of sea cucumber that are *Listeria sp., Staphylococcus sp.,* and *Rothia sp.* had antibacterial activity against these two contaminant microbes Pringgenies et al. (2018).

A consortium of bacteria is a community that had a more significant effect than a single isolate (Asri and Zulaika, 2016). Symbiotic bacteria consortium from the intestine of sea cucumber has the antibacterial capability for *B. cereus* and which will inhibit microbial contaminant which can be used as a natural preservative.

2. EXPERIMENTAL SECTION

2.1 Material

The material used in the research was the three isolate of bacteria symbion, vanname shrimp (Liptopenaeus vannamei) for experiment test, Zobell 2216 E medium, and some other chemical reagent for proximate and TVBN test. equipment used for the research is oven, furnace, Kjeldahl flask, soxhlet, incubator, Conway disk, autoclave, and laminar airflow.

2.2 Synergistic test among symbiotic bacteria

Listeria sp., Staphylococcus sp., and *Rothia sp.* from the intestine of sea cucumber tested for the synergistic test. One ose symbiotic bacteria isolate had been applied on top of each other in the Zobell 2216 E medium. The sample was incubated for 24 hours and observed. If there is no inhibition zone, the isolate was synergistic one to each other in the consortium (Asri and Zulaika, 2016).

2.3 Isolation of Secondary Metabolite

Isolate of symbiotic bacteria consortium was grown as a starter on 10 ml liquid Zobell medium in 15 ml reaction tube and shook in 120 rpm speed for 3-5 days until its reach stationary phase. Isolate the moved into 40 ml liquid Zobell media the shook for 3-5 days with 120 rpm speed. Culture of bacteria move into 50 ml centrifuge tube and centrifuge with 2800 G-force speed for 15 minutes to produce supernatant (Rau et al., 2008).

2.4 Preservation test of Bacterial Consortium Supernatant in Vanname Shrimp

The supernatant extract of the bacterial consortium was tested on vanname shrimp (*L. vannamei*). Preservation test divides into five control that is cooling (ES), bacterial supernatant (3C), sterile seawater (AS), sterile medium (MS), and without treatment (N). Vanname shrimp shocked with all treatment for 10 hours (Putra and Mirdhayati, 2009).

2.4.1 Tested Parameters

1 pH (Santoso, 2017).
2 Total Plate Count (Sitakar et al., 2016).
3 Organoleptic (SNI 01-2728.1-2006)
4 Proximate:
  Water (SNI 01-2354.2-2006)
  Ash (SNI 23541-2010)
  Fat (SNI 01-2354.3-2006)
  Protein (SNI 01-2354.4-2006).
  Karbohidrat Atma (2018).
5 Total Volatile Base-Nitrogen (TVBN) (SNI 01-2354.8-2009).

3. RESULTS AND DISCUSSION

3.1 Synergistic test Among Bacteria

Result of the symbiotic bacterial synergistic test showed no inhibition zone formed. Meaning that the three symbiotic bacteria isolate were synergistic one to each other as a bacterial consortium. Synergic bacteria to form a consortium will give optimum result than a single isolate of bacteria. The factor of bacteria isolates to form synergism to one another still unknown. Some factor could effect of synergism are 1. synergism in the nutrition synthesis, 2. Synergism among bacteria in undegraded material by other bacteria, 3. One type of bacteria has the ability to inhibit toxic compound to other bacteria (Asri and Zulaika, 2016).

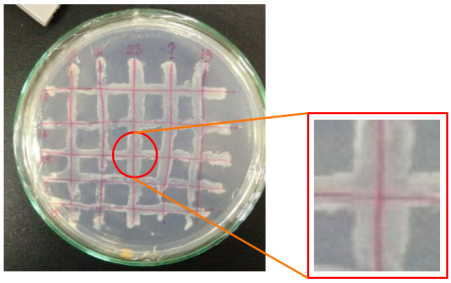

Figure 1. Synergistic test Result

3.2 pH Test on Shrimp Preservation

Value of some pH test in the shrimp preservation is on table 1. Based on pH test in shrimp preservation range from 6,46 – 7,77 where according to Purwa et al. (2012), pH ranged from 6,5 – 7,5 as a suitable pH for contaminant bacteria. pH value on Treatment on shrimp with extract was 7,44 while on positive control is 7,42.

Table 1. pH Test Result

No.	Treatment	pH
1	ES	7,42 ± 0,01
2	3C	7,44 ± 0,01
3	AS	7,10 ± 0,01
4	MS	6,46 ± 0.00
5	N	7,14 ± 0,01

According to Harsojo (2008), good pH value for food should be lower than 5,3 where the possibility of bacteria contaminant will be minimum. In reality value of pH in the shrimp product in the market range from 6,6 – 7,5.

Meaning that is neutral and nonacid for human, but it is a good condition for bacteria contaminant.

3.3 Total Colony Test of Contaminant Bacteria (TPC)

Result of total colony count in shrimp preservation as in table 2.

Table 2. Total Bacteria Count

No.	Treatment	Bacteria Colony Value (CFU/ml)
1	ES	19 x 105
2	3C	48 x 105
3	AS	114 x 105
4	MS	82 x 105
5	N	119 x 105

Result of TPC in positive control (cooling) has value 19 x 10^5 CFU/ml, while in the supernatant value is 48 x 10^5 CFU/ml. This two value is are much lower than shrimp without any treatment that is 119 x 10^5 CFU/ml. Result of TPC of all treatment is above the standard of 5 x 10^5 CFU/ml. But, according to Purwa et al. (2012) based on Directorate General of Food and Drug. Value of TPC in food product should be about 50 x 10^5 CFU/ml and above this number is not suitable for consumption. Based on result shrimp with treatment in ES and 3C is still suitable for consumption. Value of TPC is affected by the initial bacteria amount (Sukmawati and Hardianti, 2018). According to Harsojo (2008), the value of bacterial contaminant in the shrimp product in the market is 10 – 58 x 10^5. Value of negative control indicates that sterile media and sterile seawater will not effect on bacteria supernatant extract.

3.4 Organoleptic Test on Shrimp Preservation

Result of organoleptic test based on 10 respondent as in table 3. Organoleptic tests were carried out by 10 respondents with a range of values 1-9. The evaluation points observed were shrimp appearance, shrimp smell, and shrimp texture. The highest organoleptic value was obtained by ES shrimp and the lowest was by shrimp N (without treatment). Based on SNI 01-2728.1-2006 if the average organoleptic value is must be lower than 7, if the organoleptic value of the shrimp under that value, the shrimp is not suitable for consumption. Untreated shrimp (N) has a value of 5.70. So, based on the quality standards the shrimp is not suitable for consumption. While the organoleptic value of shrimp with other treatments is more than 7.

3.5 Water and Protein Content Test

The results of the water and protein content in the shrimp product shows as in Table 4. The highest value of water content is found in N shrimp with a value of 78.73% where this value is directly proportional to bacterial colonies number in

Table 3. Organoleptic Test Result

No.	Treatment	Organoleptic Value
1	ES	8,53
2	3C	7,77
3	AS	7,37
4	MS	7,43
5	N	5,70

shrimp N which is as much as 119 x 10^5 CFU / ml. The excess water content in the material will cause the material to be easily overgrown by contaminant bacteria. This is similar to Bawinto et al. (2015) the content of excess water content in a material can cause food to become rapidly damaged because it is used as a microorganisms growth's medium. Shrimp with extracts or 3C shrimp have an average water content value so that bacterial growth in shrimp 3C is minimum. However, the water content in the ES positive control was noted to be high, this was probably due to the ice melting and seeping into the shrimp meat. The lowest value of water content is found in AS shrimp where it is estimated that immersion in sterile seawater will cause the liquid in the shrimp to come out to a higher concentration. The value of protein content will also affect the number of contaminant bacteria that grow and cause the decay path faster. The highest value of protein content is found in AS shrimp. High levels of protein result in reduced water content in shrimp, this may also be a factor why the AS water content is the lowest. According to Yuarni et al. (2015), the high protein content will result in increased bacterial growth because protein is an important nutrient for bacteria. So this factor is what causes the total value of colonies in AS shrimp to be very high. The value of protein content in 3C shrimp is not much different from the value in the positive control ES shrimp where the value of both includes the average value. Overall the value of shrimp protein levels still meets the quality standard of more than 15%.

Table 4. Add caption

No.	Treatment	Water (%)	Protein (%)
1	ES	78,15	18,69
2	3C	77,77	18,45
3	AS	76,57	19,41
4	MS	78,27	17,26
5	N	78,73	17,78

3.6 Fat and Ash Content Test

The value of fat content and an ash content of shrimp in each treatment as in Table 5. Fat content in shrimp samples from three samples of ES, 3C, and AS shrimp still showed values that were suitable with existing quality standards.

According to Fendjalang (2018), the fat content in fishery products ranges from 1.00 - 1.40%. The value of fat content in MS and N shrimp is less than 1.00%, this is probably due to the high water content in these two shrimp. Khasrad and Arfal (2016) mention that the low level of fat in food caused by high water content. Contaminant bacteria will also play a role in changing the conditions of fat in a substance. The value of ash content obtained in this study shows that the ash content in MS shrimp samples exceeds the existing quality standard. The value of ash content in fishery products must be lower than 2%. According to Erni et al. (2018), high ash content can also cause by the high temperature and duration of drying in a test material.

Table 5. Fat and Ash Content Test Result

No.	Treatment	Fat (%)	Ash (%)
1	ES	1,05	0,91
2	3C	1,15	1,49
3	AS	1,10	1,67
4	MS	0,86	2,44
5	N	0,94	1,16

Table 6. Carbohydrate Content Calculation Result

No.	Treatment	Carbohydrate (%)
1	ES	1,20
2	3C	1,17
3	AS	1,25
4	MS	1,17
5	N	1,39

3.7 Carbohydrate Content Calculation

Carbohydrate content was calculated using the by difference method as in Table 6. Calculation by difference is a gross calculation that subtracts 100% with the value of other chemical content so that crude fiber will be included in the counting (Suptijah, 2012). The highest carbohydrate value is in the N sample then AS, while the ES, 3C, and MS samples are lower than that number. High carbohydrate values can be an indicator of the high growth of contaminant bacteria. According to Andarti and Wardani (2015), carbohydrates are a source of energy for the body and a simple form of carbohydrates can be used as an energy source for contaminant bacteria growth.

3.8 TVBN (Total Volatile Base-Nitrogen) Test

Result of TVBN in each treatment shows as in table 7. The results of TVBN values in shrimp are directly proportional to the total value of colonies of contaminant bacteria in shrimp as in figure 2. TVBN or Total Volatile Base-Nitrogen is a measurement of volatile material in a substance (Tapotubun and Laouhenapessy, 2008). As the graph in figure 2, the more total contaminant bacterial colonies will increase the value of TVBN in the sample. This was since contaminant bacteria will accelerate the formation of enzymes that function as protein degradation. Farahita et al. (2012) opine that the process of TVB occurs due to the work of proteolytic enzymes that break protein bonds and the result in this degradation will produce a number of volatile bases such as ammonia, H_2S, and foul-smelling trimethylamine. Enzymes roles in degradation down proteins are produced by bacterial activity.

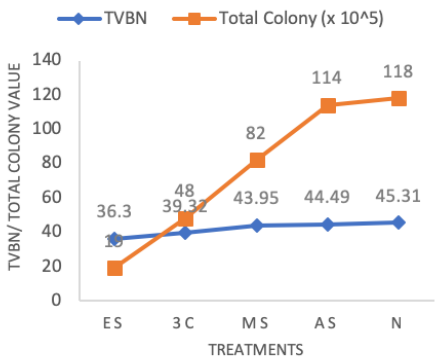

Figure 2. Comparison Between TVBN and Total Colony

Table 7. TVBN Test Result

No.	Treatment	Nilai TVB-N (mgN%)
1	ES	36,30
2	3C	39,32
3	AS	44,49
4	MS	43,95
5	N	45,31

4. CONCLUSIONS

Three isolates of symbiotic bacteria from sea cucumber *Lister sp., Staphylococcus sp.,* and *Rothia sp.* have synergic one to another. Supernatant Extract of the three bacterial consortia has a significant potential for shrimp product preservation compared to nontreatment. Result of positive control using cooling treatment still give a better result.

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